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Synthesis of alpha substituted amino acids.

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SYNTHESIS OF ALPHA SUBSTITUTED AMINO ACIDS

BY

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Approved Roger J. Thibert

A. McCurdy

K.G. Rutherford

ABSTRACT

An attempt was made to synthesize alpha phenylalanine by use of the Curtius method. This substance was to serve as a model compound for the synthesis of alpha substituted cystines. However, the material thought to be the acid azide of α-cyano-α-phenylpropionic acid could not be decomposed successfully to form the corresponding urethane.

Two alpha substituted cystines, α-ethyl and α-n-propyl were synthesized via the respective hydantoin intermediates. In connection with this preparation the synthesis of α-methyl cystine and theoretical aspects of the synthesis of alpha substituted cystines are discussed.

In the course of this study a number of new compounds were synthesized.

ACKNOWLEDGMENTS

The author wishes to express his deep gratitude and appreciation to Dr. R. J. Thibert for his guidance and his patient advice. He would also like to thank Dr. K. G. Rutherford for his willing help.

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Finally, the author wishes to express his appreciation for the advice given by his fellow students.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

The object of this study was the establishment of a feasible method for the preparation of alpha substituted cystines, for use in various subsequent investigations. Several routes to the preparation of these amino acids had been attempted before with little success in this laboratory.[1]

The synthetic route of alpha phenylalanine was to serve as a model for that of cystines having large, bulky groups in the alpha position, for instance, α-phenyl-DL-cystine. Such a method would permit the synthesis of other compounds such as α-n-butyl-DL-cystine, α-isobutyl-DL-cystine, or α-naphthyl-DL-cystine. Prior to this investigation only α-methyl-DL-cystine had been synthesized.[2,3] DL-cystine and α-methyl-DL-cystine[5] have been

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1 R. M. Ottenbrite, "Synthesis of Alpha Substituted Amino Acids." Master's thesis, Department of Chemistry, Assumption University of Windsor, 1961.

2 H. R. V. Arnstein, Biochem. J., 68, 333 (1958).

3 G. W. Kosicki, R. M. Ottenbrite, and R. J. Thibert, unpublished studies.

4 I. M. Kolthoff and D. Barnum, J. Am. Chem. Soc., 63, 520 (1941).

5 R. J. Thibert and R. M. Ottenbrite, Anal. Chem., 32, 106 (1960).

studied with the polarograph. The steric effect of the substitution of various groups in the alpha position of cystine on factors such as the half wave potential would be an interesting subject of future studies.

Cysteine and related compounds readily form chelates with copper salts. The instability constants of these resulting compounds, determined polarographically, have been shown to vary inversely with the ability of the sulfur compound to afford some protection against the effects of ionizing radiation on the cell.[6] The instability constant should vary with alpha substituted groups present. The ability of cysteine and related compounds such as cysteamine and glutathione to lower the effects of ionizing radiation has been shown.[7] Substituted cystines probably would act in a similar manner.

The metabolic behavior of alpha substituted cystines should also be interesting. Umbreit[8] found that α-methyl-DL-glutamic acid is inert to dehydrogenation and transamination, inhibits glutamine breakdown, decarboxylation, and glutamotransferase; similarly substituted cystines should also act as metabolic antagonists. The enzyme cysteine desulfhydrase catalyzes the deamination of

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6 M. M. Jones, Nature, 185, 96-7 (1959).

7 D. R. Kalkwarf, Nucleonics, 18, No. 5, 76-81, 130-1 (1960).

8 W. W. Umbreit, Symposium on Amino Acid Metabolism (Rahway, N. Y.), pp. 48-62.

cysteine by aiding in the removal of hydrogen sulfide.[9] The alpha hydrogen atom of the amino acid is removed as hydrogen sulfide in this reaction. If a larger group, which cannot be removed by the enzyme, is substituted for it, the cysteine may act as an anti-metabolite.

The reactivity of the three functional groups of cystine, the sulfhydryl group, the carboxyl group, and the amine group, should be affected by alpha substitution, particularly, if the substituent is large, Kinetic studies of reactions, e.g., the rates of saponification of esters of cystines, should therefore be of interest.

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9 C. V. Smythe, J. Biol. Chem., 142, 387 (1941).

CHAPTER II

ATTEMPTED SYNTHESIS OF ALPHA PHENYLALANINE

Introduction

The Curtius reaction has been used often by Gagnon[1] in the synthesis of α-amino acids. The acyl azide, which was prepared from the hydrazide, was decomposed in anhydrous alcohol to yield the corresponding urethane, which was then hydrolyzed to the amino acid. Attempts have been made in this laboratory[2] to synthesize α-phenylalanine by a similar procedure. However, difficulties were encountered in the decomposition of α-cyano-α-phenyl-propionylazide in reaction media such as ethanol, xylene, dimethyl formamide, and tetramethylurea. The compound decomposed in the last two liquids, but the reaction products could not be identified. In all other cases the yields were too small to bear further investigation. In this study the azide, prepared by the method of R. Ottenbrite[3] was decomposed in anhydrous alcohol containing dry hydrogen chloride gas and in trifluoroacetic acid. When practical, attempts were

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1 P. E. Gagnon et al., Can. J. Research, 25 B, 28 (1947).

2 R. M. Ottenbrite, op. cit., p. 60, 66.

3 Ibid., p. 59.

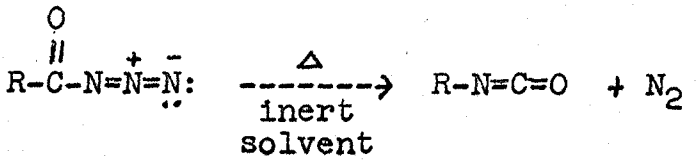
made to hydrolyse the reaction products.

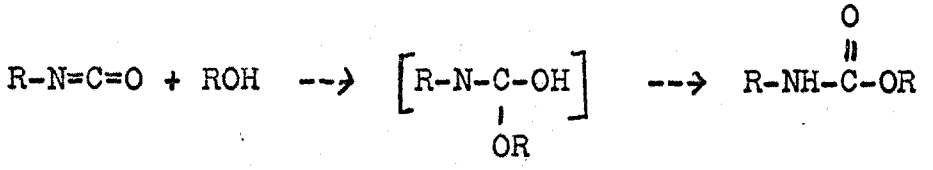
Decomposition of α-Cyano-α-Phenyl-Propionylazide

Decomposition in Alcohol

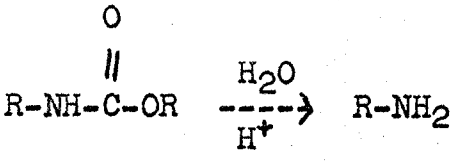
The thermal decomposition of an acyl azide in an inert solvent leads to the formation of an isocyanate.[4]

If the reaction is carried out in alcohol, the isocyanate formed reacts with the solvent to form a urethane.





Urethanes can be hydrolyzed to give the corresponding amines.



The alcohol used must be anhydrous, since water reacts readily with the isocyanate to form a carbamic acid, which decarboxylates rapidly producing a disubstituted urea.

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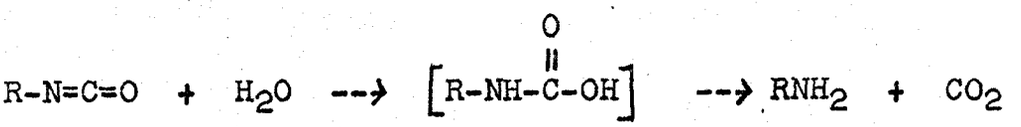
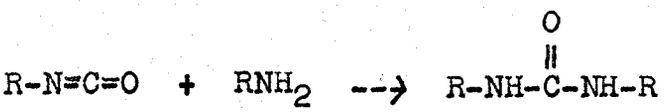
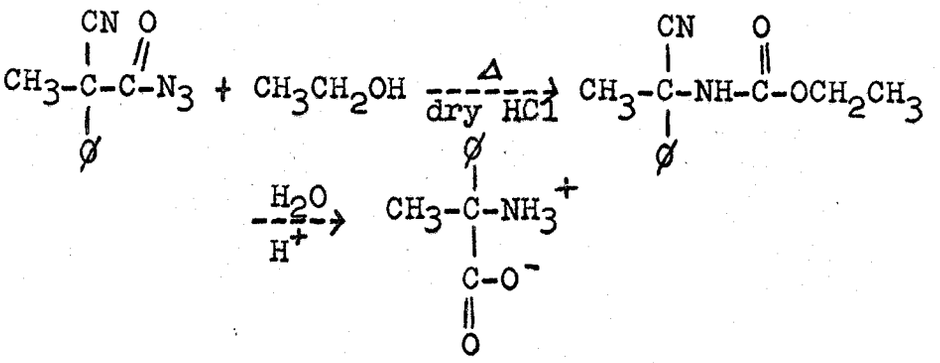
4 R. Hauser, in Organic Syntheses. Coll. Vol. 2 (New York, 1943), p. 67.





∝-Phenylalanine could therefore be prepared theoretically from the azide using the following sequence of reactions:



The dry hydrogen chloride is added as a catalyst. In the presence of water the disubstituted urea or the amino nitrile could be formed.

The azide used had been crystallized from dimethylformamide and freshly dried methanol. A crystalline, yellow solid, which decomposed at 203 - 204°, had thus been obtained. This compound was decomposed in boiling ethanol saturated with dry hydrogen chloride. The yield of gas given off during the reaction was not consistent. In some cases nearly twice the theoretical yield was observed, indicating evolution of carbon dioxide (decarboxylation).

A yellow oil was obtained, from which a white, amorphous solid that decomposed at 147 - 148° could be isolated in low yield. However, a small part of the oil could not be solidified. The analysis of the solid showed that the urethane corresponding to the azide was not obtained. The elementary analysis also excluded the carbamic acid, isocyanate, nitrile or substituted urea from consideration.

Acid hydrolysis of the solid decomposition product gave a yellowish, amorphous solid, which had many characteristics of an amino acid (see experimental section). However, the yield of this compound was very small.

Acid hydrolysis of the oil without isolation and removal of the solid, m.p. 147 - 148° (dec.), gave a product similar to that mentioned above. The melting point of this solid is the same as that reported in the literature for α-phenylalanine.[5] However, the yield was again very small.

Since the decomposition product, m.p. 147 - 148° (dec.), could not be identified, and since its yield and that of the hydrolysis product were very small resulting in an overall yield of suspected amino acid of less than ten percent, this phase of the study was terminated. (The main purpose of the synthesis of α-phenylalanine had been the determination of a convenient method of preparation of alpha substituted cystines having large, bulky groups in the alpha position.)

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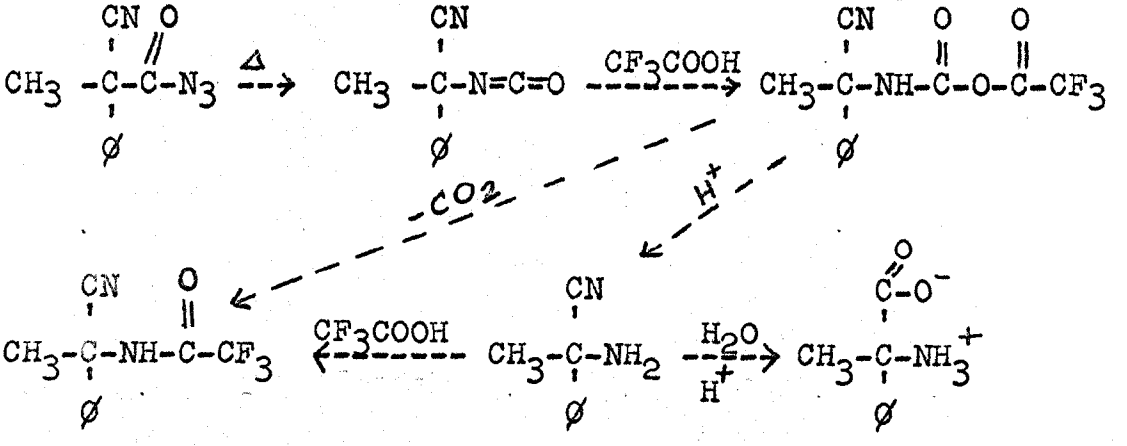
5 Beil. XIV, p.507.

When the decomposition of α-cyano-α-phenyl-propyonyl-azide in ethanol proved unsuccessful, trifluoroacetic acid, a very strong organic acid, was used instead of the alcohol.

Trifluoroacetanilide was prepared in this laboratory by the decomposition of benzazide in trifluoroacetic acid. This amide has also been prepared by heating aniline in trifluoroacetic acid at 120 - 170° for several hours.[6] It seems likely, therefore, that an amine is an intermediate in the decomposition of benzazide in trifluoroacetic acid.

Similar reactions could be expected in the case of α-cyano-α-phenyl-propionylazide. The isocyanate would be formed first; this reacts with the acid to produce the corresponding mixed anhydride. The latter could either decarboxylate giving the amide, or, more likely (see above) be hydrolyzed forming the amine. The amine could then react with another equivalent of acid to give the amide; hydrolysis of either the amine or the amide would produce the amino acid.



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6 Beil. XII_2, p. 141.

In the preparation of benzazide from ethyl benzoate the procedure used by R. Ottenbrite[7] was followed. The melting point of trifluoroacetanilid agrees with that reported in the literature.[8]

Several modifications of one basic method were adopted in the decompositon of α-cyano-α-phenyl-propionylazide in trifluoroacetic acid. The azide was added to the acid at room temperature; the gas evolved in the exothermic reaction was collected in a graduated cylinder filled with limewater, The solution was allowed to stand at room temperature for one hour and then poured on ice. The yellow oil obtained was separated from the water, extracted with ether, neutralized with sodium carbonate, and dried over sodium sulfate. Attempts to isolate a solid from this oil were unsuccessful. The amount of gas collected was quantitative.

In a modified method the solution was allowed to stand at room temperature overnight after the decomposition; from the residue a white solid, m.p. 186°, was obtained by addition of ether. The yield of this solid, however, was small. Elementary analysis showed the absence of fluorine. The composition of the compound agreed with that of α-cyano-α-phenyl propionylhydrazide. A mixed melting point determination confirmed this agreement. Solubility character-

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7 R. Ottenbrite, loc. cit.

8 R. Reed, J. Am. Chem. Soc., 78, 801 (1956).

istics also agreed. After this solid had been filtered off, another white solid of m.p. 122 - 123° could be isolated from the ethereal solution. However, the yield of this compound was very small; its infrared absorbtion spectrum indicated the absence of fluorine. Since the yield was so small, no attempts were made to identify this compound.

The same major decomposition product of m.p. 186° was obtained when trifluoroacetic acid plus its anhydride or the acid plus a small amount of water were used. Heating at reflux temperature of the azide in trifluoroacetic acid for twenty hours gave the same result.

Experimental

Decomposition of α-Cyano-α-Phenyl-Propionylazide in Ethanol

The ethanol was dried according to the method of Lund and Bjerrum.[9] The hydrogen chloride used was passed through concentrated sulfuric acid and over anhydrous calcium chloride.

Dry azide (5 g.) was added to freshly dried ethanol (35 ml.) saturated with dry hydrogen chloride in a 100 ml. round bottom flask equipped with a reflux condenser and connection to 1000 ml. graduated cylinder filled with lime-water. The alcohol was heated rapidly to the boiling point

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9 R. Lund and J. Bjerrum, Ber., 64, 210 (1931).

with a bunsen burner flame. Gas evolution was rapid; and the light yellow mixture decolourized quickly. All of the azide had reacted within five minutes. A total of 573 ml. of a colourless gas were collected. (This amount corresponds to a quantitative evolution of gas.) The lime-water showed only slight cloudiness, indicating that at most only a small amount of carbon dioxide had been given off.

The reaction mixture was allowed to cool to room temperature, the nearly colourless liquid was put on the solvent stripper, and the ethanol was removed by evaporation.

The yellow oil obtained was solidified by the addition of chloroform and petroleum ether; this procedure gave 2.5 g. of a white solid, m.p. 143 - 148° (dec.). Crystallization from hot ethanol produced 2.1 g. of a white solid, m.p. 147 - 148° (dec.).

Anal. Calcd. for the urethane $C_{12}H_{14}O_2N_2$:
C,66.00; H,6.44; N,12.89.
Found: C,53.14; H,5.31; N,18.09.

α-Phenylalanine

The solid product of the decomposition of the azide, (1.5 g.), was heated at reflux temperature with 20% hydrochloric acid (60 ml.) for 110 hrs. The solution, which had turned cloudy after a few hours, was then evaporated *in vacuo* to nearly complete dryness. Water (10 ml.) was added, followed by drops of 10% ammonium hydroxide solution until a white, flocculant precipitate formed throughout the

liquid. The latter was filtered off. After drying of the precipitate at room temperature a light brown solid (0.3 g.) was obtained. It was found to be slightly soluble in water and soluble in dilute sodium hydroxide and dilute hydrochloric acid. The solid started to char at 260° in agreement with the value found in the literature.[10]

The following modification of the above procedure was also used: The oil obtained by the decomposition of azide (5 g.) in ethanol (35 ml.) carried out as above, was heated at reflux temperature with 20% hydrochloric acid (40 ml.) for fifty hours. The liquid was evaporated in vacuo and the syrupy residue obtained dissolved in water (40 ml.); 5 M. ammonium hydroxide was added dropwise with stirring until a curdy, white precipitate appeared throughout the liquid at a pH of about five; and the solid was filtered. The light grey material started to char at 260°. It was soluble in dilute acid and base and slightly soluble in water. The yield was 0.6 g.

Decomposition of α-Cyano-α-Phenyl Propionylazide in Trifluoroacetic Acid

In a 50 ml. three-necked round bottom flask equipped with a reflux condenser and connection to a 500 ml. graduated cylinder filled with limewater, dried azide (0.5 g.) was added slowly over a period of five minutes to trifluoroacetic acid (10 ml.). The flask heated rapidly; a gas

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10 Beil. XIV, p. 507.

was given off quickly while the yellow mixture lightened in colour. A quantitative amount of gas (57 ml.) was collected. The limewater clouded up only slightly. After one hour the solution was poured on ice ; and as a result a yellow oil was obtained, which was extracted with ether (20 ml.), neutralized with sodium carbonate, and dried over sodium sulfate. The ether was evaporated in a solvent stripper leaving a yellow oil behind, which could not be solidified despite numerous attempts to do so.

Modified Procedure

Azide (10 g.) was added slowly to trifluoroacetic acid (25 ml.). Gas (1150 ml.) was evolved rapidly and collected as above and the reddish solution so produced was allowed to stand at room temperature overnight in an evaporating dish. A few millilitre of ether were added to the syrupy, clear, light yellow residue; and the mixture was stirred for a few minutes until a light yellow precipitate appeared. The mixture was refrigerated for several hours and 3.5 g. of a solid, m.p. 186°, were filtered off. The compound was soluble in dilute acid and base and acetone, but insoluble in chloroform and water. It was crystallized from ethanol.

Anal. Calcd. for $C_{11} H_9 O N F_3$:

C,54.52; H,3.74; N,11.62; F23.52.

Found: C,63.59; H,5.93; N,22.11; F,0.

A white solid, m.p. 122 - 123°, was obtained when the

ether was allowed to evaporate from the filtrate. The infrared absorbtion spectrum of this solid showed the absence of fluorine. Since the yield was very small, the identification of this solid was not attempted.

Other reaction conditons such as the use of a mixture of the acid and its anhydride, the presence of 10% by volume of water, or the heating under reflux of the reactants did not influence the nature of the main final product.

Trifluoroacetanilide

Benzazide (2.5 g.) was heated at reflux temperature with trifluoroacetic acid (10 ml.) for 24 hrs. When the reddish solution was poured on cracked ice, a white solid precipitated instantly. The mixture was filtered and the solid was washed several times with distilled water. This procedure gave 2.6 g. (70%) of a final product of m.p. 89 - 90°.

Discussion and Conclusions

An attempt was made to synthesize α-phenylalanine by the decomposition of α-cyano-α-phenyl-propionyl azide in ethanol, saturated with dry hydrogen chloride, or trifluoroacetic acid and acid hydrolysis of the decomposition products. When ethanol was used a compound which is very probably the amino acid desired was obtained. However, since all yields were very small and difficulties were encountered in identifying some reaction products, the study

was terminated.

The fact that the solid decomposition product of the reaction in ethanol can be further hydrolyzed to an amino acid strongly suggests that an amine, urethane, or substituted urea is involved. However, the elementary analysis does not support any of these compounds. In the case of the urea compound the percentage of hydrogen and nitrogen agrees with that reported, but the carbon content is too high. The possibility exists that a mixture was obtained.

The successful synthesis of trifluoroacetanilide by the decomposition of benzazide in trifluoroacetic acid suggested a similar synthesis in the case of α-cyano-α-phenyl-propionylazide. The fact that trifluoroacetanilide has been prepared from aniline and trifluoroacetic acid suggests the amino nitrile as an intermediate. It was hoped that either the amino nitrile or the amide could be isolated. However, the hydrazide as the reaction product cannot be explained at this time. The azide gave the following elementary analysis: C,59.6; H,5.1; N,23.2. The analysis calculated for $C_{10}H_8ON_4$ is: C,59.90; H,4.02; N,28.08. It was first thought that some degree of decomposition could have occurred during crystallization from dimethyl formamide and methanol. However, the hydrazide as the main decomposition product may indicate the possibility that the azide was not obtained in the first place. It is unlikely that the hydrazide was introduced as an impurity in the azide, since the former is very soluble in the acidic

reaction medium used for the preparation of the azide. Moreover, the amount of gas collected was quantitative.

It was not thought practical during this particular study to work on the identification of the minor product of the decomposition of the azide in trifluoroacetic acid. However, from the point of view of the mechanism involved this might be rewarding. The reactions involved in both ethanol and trifluoroacetic acid should be worth further study. It is suggested that the synthesis of the azide from the hydrazide be modified and that all reaction products be identified.

CHAPTER III

THE SYNTHESIS OF ALPHA SUBSTITUTED CYSTINES

Previous Syntheses of α-Methyl-DL-Cystine

A survey of the literature has indicated that the only alpha substituted cysteines or cystines prepared previous to this work are α-methyl-DL-cystine, S-benzyl-α-methyl-DL-cysteine and α-methyl-DL-cysteine hydrochloride. These have been synthesized either by the Strecker synthesis or by use of the Bucherer reaction.[1,2,3,4]

In 1955 Potts prepared S-benzyl-α-methyl-DL-cysteine (from which α-methyl-DL-cystine can be synthesized using sodium and liquid ammonia). The author reacted benzylmercaptoacetone (prepared according to Wahl[5]) with potassium cyanide and ammonium carbonate in 50% alcohol to form 5-benzylmercaptomethyl-5-methyl-hydantoin (I). Heating at reflux temperature of (I) with barium hydroxide solution and precipitation of barium ions as barium carbonate from

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1 H. R. V. Arnstein, Biochem. J., 68, 333 (1958).

2 R. Ottenbrite, G. W. Kosicki, R. J. Thibert, op. cit., pp. 6-13.

3 I. W. Stapleton and J. M. Swan, Australian J. Chem., 13, 416 (1960).

4 K. T. Potts, J. Chem. Soc., 1632 (1955).

5 R. Wahl, Ber., 55, 1449 (1922).

the solution at 100° gave S-benzyl-α-methyl-DL-cysteine (II, 71%).

Stapleton[6] synthesized S-benzyl-α-methyl-DL-cysteine according to the method of Potts.[7] From (II) he obtained α-methyl-DL-cystine (III) as follows: He treated (II) with metallic sodium in liquid ammonia and, after evaporation of the ammonia, dissolved the residue in water, extracted it with ether, and aerated it for two days. The solution was made just acid and applied to a column of "Zeakarb 225" resin in the H^+ form. The chloride was removed with water and the amino acid (74%) was eluted with 1 N. ammonium hydroxide. The author also prepared α-methyl-DL-cysteine hydrochloride from (II) by working in an oxygen-free medium and dissolving (II) in dilute hydrochloric acid.

Arnstein[8] prepared the amino nitrile of S-benzyl-mercaptoacetone by shaking overnight a mixture of the ketone, ammonium chloride, ammonium cyanide, concentrated ammonium hydroxide, and ethanol, saturated with gaseous ammonia. The crude amino nitrile was hydrolyzed by heating it at reflux temperature with concentrated hydrochloric acid. The residue after evaporation was dissolved in water and the pH was adjusted to 6 when S-benzyl-α-methyl-cysteine (70%) crystallized.

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6 I. W. Stapleton, op. cit., p. 420.

7 K. T. Potts, op. cit., p. 1633.

8 H. R. V. Arnstein, op. cit., p. 335, 337.

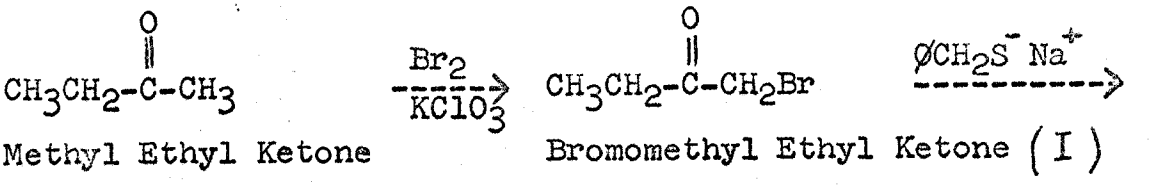
Reduction of the S-benzyl group by sodium in liquid ammonia, oxidation of the residue by air at a pH of 8 in the presence of a trace of ferric chloride, and evaporation to a small volume at a pH of 6 produced (III) in 50% yield.

Shortly after Arnstein's work the synthesis of this substituted cystine by the Strecker method was confirmed in this laboratory.[9]

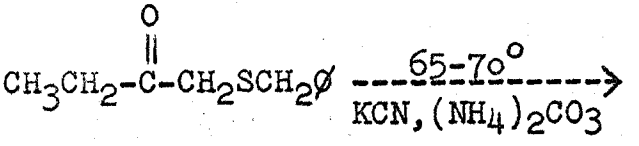
Theoretical Considerations

In this study ∝-ethyl-DL-cystine and ∝-n-propyl-DL-cystine were prepared according to the following reaction schemes:

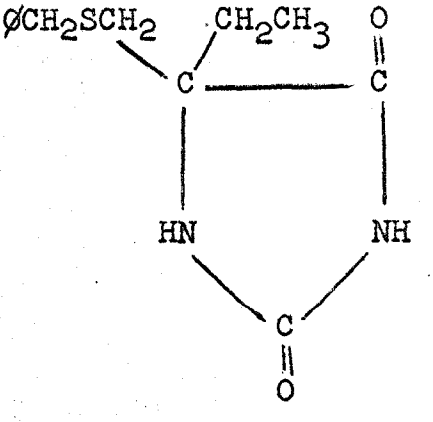
∝-Ethyl-DL-Cystine



Methyl Ethyl Ketone — Bromomethyl Ethyl Ketone (I)



Benzylmercaptomethyl Ethyl Ketone (II)



5-Benzylmercaptomethyl-5-Ethyl Hydantoin (III)

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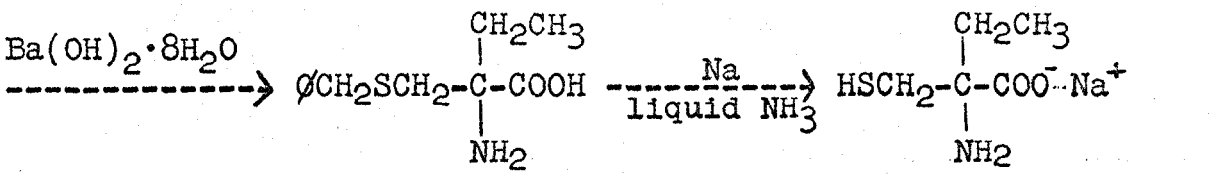
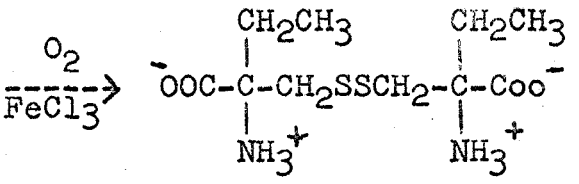
9 G. W. Kosicki, R. M. Ottenbrite, and R. J. Thibert, op. cit., p. 13.



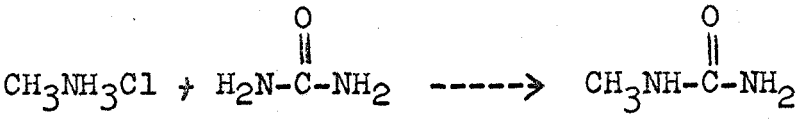
S-Benzyl-α-Ethyl-DL-Cysteine (IV)

α-Ethyl-DL-Cysteine Sodium Salt

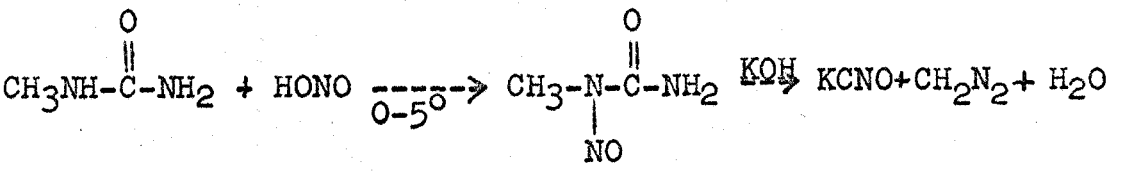


α-Ethyl-DL-Cystine (V)

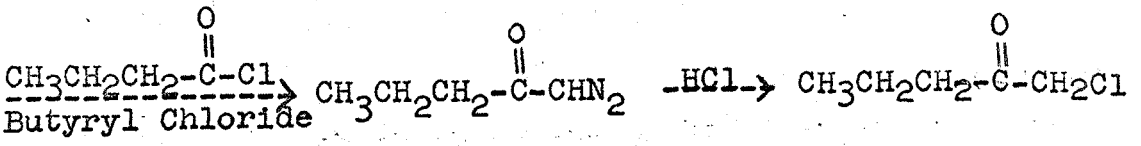
α-n-Propyl-DL-Cystine



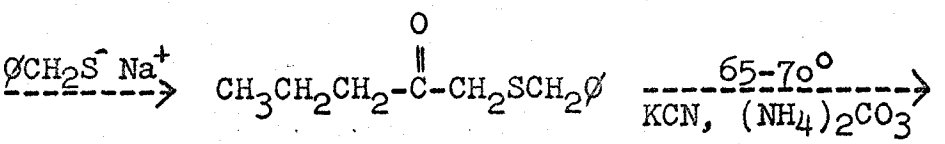
Methylamine Hydrochloride — Urea — N-Methyl Urea



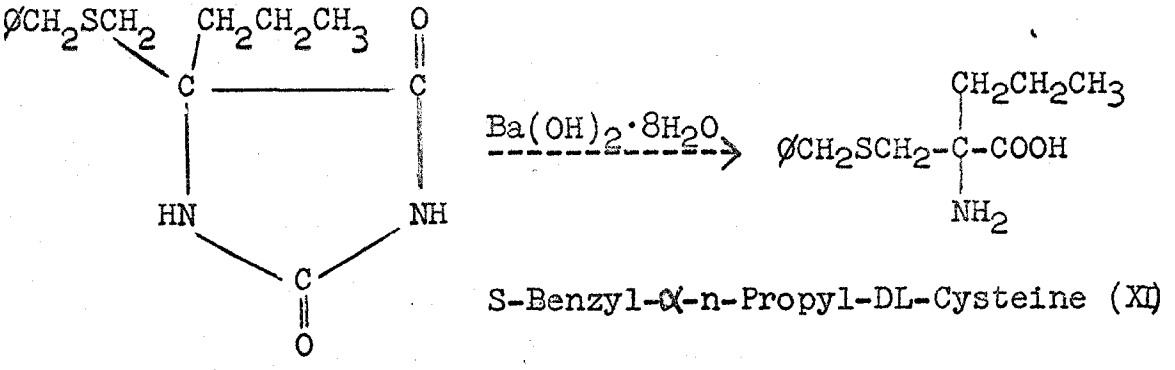
Nitrosomethylurea (VI) — Diazomethane (VII)



n-Propyl Diazomethyl Ketone — Chloromethyl n-Propyl Ketone (VIII)

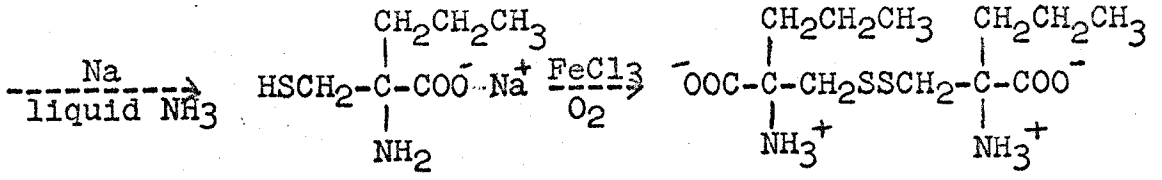


Benzylmercaptomethyl n-Propyl Ketone (IX)



5-Benzylmercaptomethyl
-5-n-Propyl Hydantoin (X)

S-Benzyl-α-n-Propyl-DL-Cysteine (XI)



α-n-Propyl-DL-Cysteine
Sodium Salt

α-n-Propyl-DL-Cystine (XII)

Preparation of α-Haloketones

Introduction

Chloromethyl ethyl ketone has been prepared by the action of chlorine or sulfurylchloride on methyl ethyl ketone,[10] by the oxidation of 1 chloro-butanol (2) with chromic acid,[11] or by heating 4,4 dimethyl-2-chloromethyl-2-ethyl -1,3 dioxolon (5) with acetic acid and hydrochloric acid at 100°.[12] J. R. Catch synthesized bromomethyl ethyl ketone by the addition of bromine to methyl ethyl ketone in the presence of water and potassium chlorate[13] and by the reaction of propionyl bromide with diazomethane and decomposition of the resulting diazoketone with hydrobromic acid.[14]

Bromomethyl or chloromethyl n-propyl ketone has been synthesized similarly by the reaction of the acid bromide or acid chloride of propionic acid with diazomethane and decomposition of the resulting diazo-ketone with hydrochloric or hydrobromic acid.[15,16,17] The ketone has also been prepared by the bromination of methyl n-propyl ketone

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10 Beil. II, p. 507.

11 Ibid., II, Vol. I, p. 731.

12 Ibid., I, Vol. I, p. 348.

13 J. R. Catch, op. cit., p. 272.

14 Ibid., p. 278.

15 E. B. Reid, J. Org. Chem., 16, 1566 (1951).

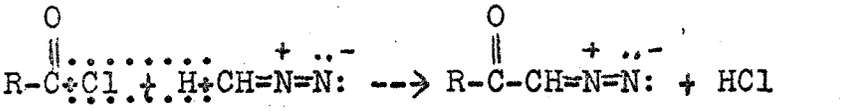
16 R. D. Haworth, J. Chem. Soc., 3617 (1954).

17 J. R. Catch, op. cit., p. 276.

in the presence of water and potassium chlorate.[18]

In the bromination of ketones by bromine, the bromonium ion formed by the addition of bromine to the enol form of the ketone, is an intermediate.[19] The formation of the enol is catalyzed by acid. Substitution of alkyl groups for α-hydrogen atoms accelerates halogenation and α-halo substituents retard it in acid catalyzed halogenation.[20] The C=C bond is stabilized by hyperconjugation due to alkyl groups. Breakage of α C-H bonds is facilitated in the presence of acid when the carbonyl oxygen atom is protonated resulting in an electron shift.[21] Thus in the bromination of methyl ethyl ketone in water bromination occurs preferably at the number three carbon atom. As a result more of the isomer 3-bromo-2-pentanone is produced than 1-bromo-2-pentanone. The addition of potassium chlorate allows the regeneration of bromine from potassium bromate and the bromide anion.

Acid chlorides condense readily with diazomethane to give the corresponding diazoketones.



Diazomethane is conveniently prepared as an ethereal

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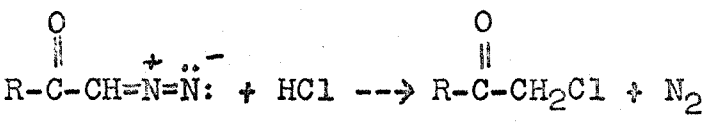
18 J. R. Catch, op. cit., p. 278.

19 E. S. Gould, Mechanism and Structure in Organic Chemistry (New York, 1960), p. 521.

20 Ibid., p. 383.

21 Ibid., p. 374.

solution from nitrosomethylurea.[22,23] The diazoketone is decomposed with mineral acid to the α-haloketone.[24]



The diazoketone does not need to be isolated in this reaction. The preparation of the diazoketone must be made under anhydrous conditions, since the acid chloride reacts with water to form the corresponding acid.

α-Haloketones readily undergo bimolecular nucleophilic substitution reactions.[25] They decompose slowly in the presence of small amounts of water, for instance, losing HCl or HBr to form the corresponding alcohol; stabilizing agents such as magnesium oxide are therefore added in small amounts.[26]

The Condensation of α-Haloketones with α-Toluenethiol

The sodium salt of α-toluenethiol is formed easily by extracting the latter with sodium hydroxide solution. It can then be condensed with α-haloketones to produce sulfur

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22 F. Arndt, in Organic Synthesis, ed. A. H. Blatt (New York, 1957), Coll. Vol. 2, p. 461.

23 Ibid., p. 165.

24 L. F. Fieser and M. E. Fieser, Advanced Organic Chemistry (New York, 1961), p. 386.

25 D. J. Cram and G. S. Hammond, Organic Chemistry (New York, 1959), p. 240.

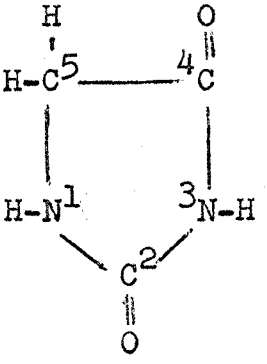
26 J. R. Catch, op. cit., p. 273.

containing ketones in which the sulfur group is protected from oxidation. The benzyl group can be removed conveniently by reduction with sodium in liquid ammonia.

Preparation and Hydrolysis of Hydantoins

Introduction

Baeyer isolated hydantoin in 1861 as one of the reduction products of allantoin during his study of uric acid.[27] The structure of this cyclic ureide was first proposed by Strecker[28] to be:



Methods of Synthesis of Hydantoins

Several ways of preparing hydantoins have been used, such as the reaction of:

(1) α-Amino acids with potassium cyanate.[29,30]

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27 A. Baeyer, Ann., 117, 178 (1861).

28 A. Strecker, Ann, 155, 177 (1870).

29 F. Urech, Ann., 165, 99 (1873).

30 E. Ware, Chem. Revs., 46, 406 (1950).

(2) α-Amino acid amides[31] or nitriles[32] with potassium cyanate.

(3) Amino acids and alkyl or aryl isocyanates.[33]

(4) Amino acids and urea or derivatives of urea.[34]

(5) Urea and α-hydroxy acids or α-hydroxy nitriles.[35]

(6) Urea and α-dicarboxyl compounds.[36]

(7) Urea and unsaturated acids.[37]

(8) Amino acids and urethanes.[38]

(9) Isocyanates derived from amino acids and amines.[39]

(10) Amino acid esters or amides and alkyl chloroformates.[40]

(11) Chloroacetyl urethane and amines.[41]

(12) Bromoacetylurea.[42]

(13) Cyanoacetamides and alkali hypohalides.[43]

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31 T. B. Johnson and B. H. Nicolet, J. Am. Chem. Soc., 36, 355 (1914).

32 W. T. Read, J. Am. Chem. Soc., 44, 1746 (1922).

33 E. Ware, op. cit., p. 411.

34 Ibid., p. 413.

35 Ibid., p. 414.

36 Ibid., p. 415.

37 Ibid., p. 416.

38 O. Diels, et al., Ber., 38, 297, (1905).

39 E. Ware, op. cit., p. 417.

40 Ibid., p. 420.

41 Ibid., p. 421.

42 Ibid., p. 422.

43 Ibid.

(14) Carbonyl compounds, potassium cyanide, and ammonium carbonate (Bucherer-Bergs Synthesis).[44]

C-5 unsaturated hydantoin derivatives, which may be reduced by a number of common reducing agents, can be obtained by condensation of a hydantoin with a number of carbonyl compounds.[45] C-5 substituted hydantoins can be hydrolysed to amino acids.

The Bucherer-Bergs Synthesis

This method of synthesis involves the reaction of aldehydes or ketones with potassium cyanide and ammonium carbonate in 50% alcohol giving 5-C substituted hydantoins. The preparation works well for many carbonyl compounds except formaldehyde, some hydroxy-and nitroso-aryl aldehydes, certain unsaturated aldehydes, and pyruvic acid. Bergs[46] prepared a number of 5-substituted hydantoins by treating a ketone or aldehyde with potassium cyanide, ammonium carbonate, and carbon dioxide under several atmospheres of pressure, at a temperature of 80° for 4-6 hrs. The following mechanism was suggested for the reaction:

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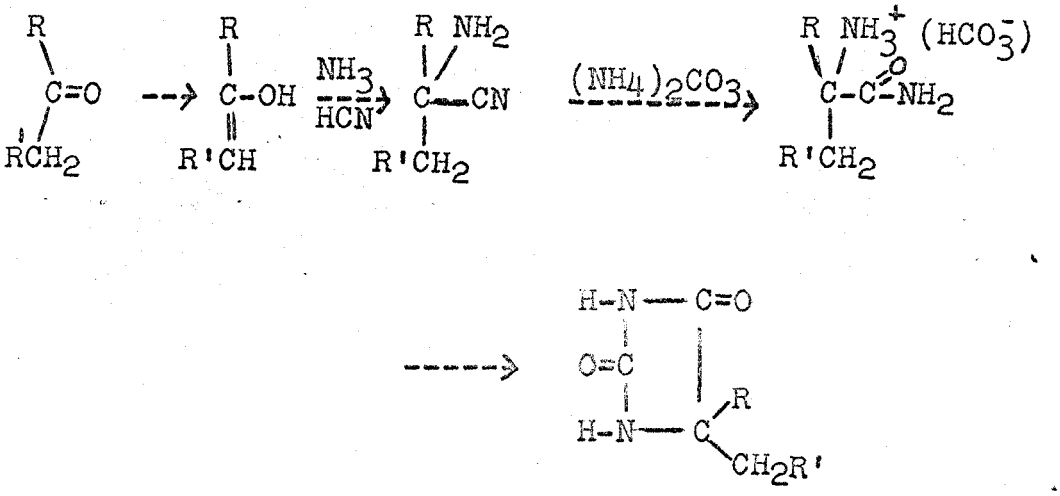
44 E. Ware, op. cit., p. 422

45 Ibid., p. 431-36.

46 H. Bergs, German pat. 566,094. (c.f. C.A., 27, 1001 (1933)).

47 K. Slotta, et al., Ber., 67, 1529 (1934).



Bucherer[48] found that a cyanohydrin reacted with ammonium carbonate to give a hydantoin. The reaction took place at a temperature not higher than 60 - 70°; the use of carbon dioxide under pressure was not necessary. He also observed[49] that cyanohydrins react equally well with ammonium carbamate to form hydantoins; α-amino nitriles were also found to give nearly quantitative yields of hydantoins when treated with carbon dioxide in aqueous solution. The following mechanism was suggested:

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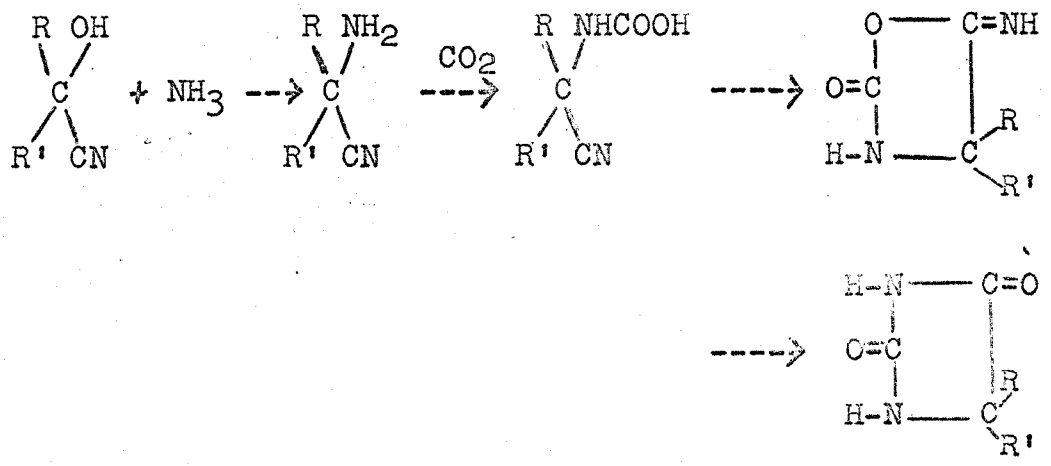
48 H. T. Bucherer and H. Barsch, J. pract. Chem., 140, 151 (1934).

49 H. T. Bucherer and W. Steiner, J. prakt. Chem., 140, 291 (1934).



Hydrolysis of Hydantoins to Form Amino Acids

Prolonged heating of hydantoins with a large excess of barium hydroxide in aqueous solution yields the corresponding amino acids. This synthesis was first suggested as a general method of preparation of amino acids by Wheeler and Hoffman.[50] It has now been used for many amino acids.[51,52,53] Instead of metallic hydroxides aqueous ammonium sulfide,[54] concentrated hydrochloric acid,[55] 60% sulfuric acid,[56] and acetic acid and hydrogen

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50 H. L. Wheeler and C. Hoffman, Am. Chem. J., 45, 368 (1911).

51 E. Ware, op. cit., p. 443.

52 H. C. White, U. S. 557,920 (1951).

53 G. Nadeau, R. Gaudry, Can. J. Research, 27B, 421 (1949).

54 W. J. Boyd and W. Robson, Biochem. J., 29, 546 (1935).

55 F. Urech, Ann., 164, 255 (1872).

56 H. T. Bucherer and W. Steiner, loc. cit.

chloride[57] has been used in the hydrolysis.

Removal of the Benzyl Group with Sodium in Liquid Ammonia

The use of sodium in liquid ammonia as a reducing medium is quite common. Many organic compounds have been reduced this way.[58]

In liquid ammonia, alkali metals dissociate into positive ions and electrons; the reducing power of the medium is due to these electrons. In many cases the reducing action involves bond rupture and the combination of an electron with each of the resulting fragments.[59]

$$R_{n-1}A\text{-}A'R'_{n'-1} + 2e \longrightarrow [R_{n-1}A]^- + [R'_{n'-1}A']^-$$

where n is the valence of element A.

This type of mechanism occurs in the removal of the benzyl group from S-benzyl substituted amino acids; in this case ϕCH_2^- and ^-SR are formed. On addition of acid the sulfhydryl compound RSH is generated. This type of reaction has been used quite frequently in the preparation of amino acids and related compounds.[60]

After removal of the S-benzyl group, cysteines can be oxidized by atmospheric oxygen to cystines. The oxidation process is enhanced by the use of such agents as

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57 J. R. Catch, et al., J. Chem. Soc., 1609 (1947).

58 G. W. Watt, Chem. Revs., 46, 317-79 (1950).

59 Ibid., p. 322.

60 G. W. Watt, op. cit., p. 350.

trivalent iron.[61]

Experimental

The Synthesis of ∝-Ethyl-DL-Cystine

Bromomethyl Ethyl Ketone (I)[62]

Methyl ethyl ketone (2500 ml., Fisher certified reagent) was dried over anhydrous calcium chloride overnight, filtered, and fractionally distilled using a short Vigreux column. The fraction of b.p. 78.5 - 80.5° was collected.

Bromine (200 ml. - 630 g., 99.5% purity) was added dropwise to a mechanically stirred mixture of methyl ethyl ketone (630 ml. - 500 g.), potassium chlorate (120 g.), and water (1000 ml.) over a period of five hours. To initiate the reaction the temperature of the reaction mixture was raised to 50°. The reaction then proceeded smoothly at 30 - 40°.

After the addition of bromine had been completed, the mixture was allowed to stand at room temperature overnight; the heavy layer of haloketones was separated from the aqueous phase, shaken with magnesium oxide (2 g.) and water (50 ml.), separated from the water, and dried over anhydrous calcium chloride for five hours. The nearly colourless, very lachrymatory liquid (968 g.), which was obtained, was distilled at 150 mm. pressure; the fraction distilling

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61 H. A. Krebs, Biochem. Z., 204, 322 (1929).

62 J. R. Catch, et al., J. Chem. Soc., 272 (1948).

over at 70 - 110° was collected. This liquid was then fractionated carefully. The column used contained a packed section, 120 x 1.4 cm., of 5 mm. inside diameter pieces of glass tubing, about 1 cm. long; it was insulated with a 1 cm. thick lagging of asbestos and had a total condensation, partial take-off head. The fraction (60 g.) of nearly colourless liquid distilling over at 103 - 105° (150 mm.) was collected in a receiver containing a few milligram of magnesium oxide. The product became light green on standing for a week in the refrigerator.

The derivative prepared was anilinomethyl ethyl ketone; white plates, m.p. 81° - reported[63] 81°.

Benzylmercaptomethyl Ethyl Ketone (II)[64,65]

α-Toluenethiol (Eastman Organic Chemicals, 143 g. - 135 ml.) in ether (250 ml.) was extracted with 10% sodium hydroxide solution (400 g.). The mixture was shaken periodically and left standing for two hours. The aqueous phase was then separated, heated at reflux temperature with bromomethyl ethyl ketone (82 g.) for 2.5 hours, and allowed to stand at room temperature overnight. A medium brown liquid layer appeared on top of a yellow, aqueous phase and was separated. The liquid was extracted with water until the washings contained no bromide ion as shown by

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63 J. R. Catch, loc. cit.

64 R. Wahl, Ber. 55, 1449 (1922).

65 K. T. Potts, J. Chem. Soc. 1632 (1955).

the silver nitrate test. A deep yellow oil was obtained, which was dried over anhydrous sodium sulfate overnight.

The boiling point is 115 - 130° (3.5 mm.). A second distillation gives b.p. 126 - 128° (3.5 mm.). The yield is 94.5 g. (98%).

Anal. Calcd. for $C_{11} H_{14} O S$:

C,68.02; H,7.23; S,16.51.

Found: C,68.13; H,7.19; S,16.28.

The semicarbazide of the ketone shows m.p. 85 - 92°. After one crystallization from 50% ethanol the compound melts at 100 - 101°.

5-Benzylmercaptomethyl-5-Ethyl Hydantoin (III)[66,67]

In a 300 ml. three-necked round bottom flask, equipped with a reflux condensor and mechanic stirrer, benzylmercaptomethyl ethyl ketone (30 g.), potassium cyanide (13 g., Fisher certified reagent), and ammonium carbonate (44 g., Fisher certified reagent) in fifty percent ethanol (120 ml.) were heated on a steam bath kept at 65 - 70° for seven hours. At this temperature the ammonium carbonate decomposed rapidly giving a light yellow solution. At the end of the reaction time two liquid layers were observed, a brown-yellow top layer and a smaller volume of light yellow liquid on the bottom.

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66 K. T. Potts, loc. cit.

67 I. W. Stapleton and J. M. Swan, Austral. J. Chem., 13, 416 (1960).

The mixture was allowed to stand at room temperature for forty hours. A large cake of light yellow, crystalline solid appeared. This was poured on about 100 ml. of ice water, filtered with suction, and washed with small amounts of ether.

After drying *in vacuo* the yield of needles of a white solid, m.p. 125 - 128°, is 36.8 g. or 90%. Upon crystallization from hot ethanol white needles, m.p. 130 - 131° (32.4 g.) are obtained.

The hydantoin is soluble in 10% sodium hydroxide solution, absolute ethanol, chloroform, and acetone, but not in 10% hydrochloric acid, water, or ether.

Anal. Calcd. for $C_{13}H_{16}O_2N_2S$:
C,59.03; H,6.10; N,10.65; S,12.12.
Found: C,59.09; H,6.05; N,10.40; S,11.85.

S-Benzyl-α-Ethyl-DL-Cysteine (IV)[68]

5-Benzylmercaptomethyl-5-ethyl hydantoin (30 g.), barium hydroxide (Fisher certified reagent, 142 g.), and water (750 ml.) were heated at reflux temperature for forty-two hours. Carbon dioxide gas, generated from marble chips and 6 N hydrochloric acid, was then passed into the mixture at 100° until a filtered sample of the above mixture did not give a positive test for the barium ion with 10% sulfuric acid. The hot mixture was then filtered, the filter

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68 K. T. Potts, *loc. cit.*

cake was washed with hot water and acetone, and the filtrate was allowed to cool to room temperature, whereupon seven gram of unreacted hydantoin precipitated and were filtered from the mixture. A very light yellow solid (23 g.), m.p. 217 - 220°, precipitated when the solution of S-benzyl amino acid was allowed to stand at room temperature overnight. After one crystallization from hot water, 20 g. (74%) of a crystalline, white powder, m.p. 224 - 226°, were obtained. The ninhydrin test gave a reddish-purple colour. The test for the sulfhydryl group using sodium nitroprusside was negative. The solid is soluble in 10% sodium hydroxide solution and 10% hydrochloric acid; it is partly soluble in hot water, but insoluble in cold water, ethanol, acetone, and chloroform.

Anal. Calcd. for $C_{12}H_{17}O_2NS$:
C,60.20; H,7.15; N,5.88; S,13.31.
Found: C,60.50; H,7.00; N,5.93; S,13.64.

α-Ethyl-DL-Cystine (V)[69,70]

Sodium (3.3 g., Fisher certified reagent) cut into small pieces and freshly dried S-benzyl α-ethylcysteine (12 g.) were added alternatively in small quantities, while stirring, over a period of thirty minutes to liquid ammonia (about 200 ml.) in a one litre three-necked round

69 H. R. V. Arnstein, Biochem. J., 68, 333 (1958).

70 J. L. Wood and V. du Vigneaud, J. Biol. Chem., 130, 109 (1939).

bottom flask provided with a mechanical stirrer and reflux condensor. After the addition of a few pieces of sodium the solution turned blue for a short time and remained so for fifteen minutes after all the sodium had been added. The blue colour was discharged with about 250 mg. of ammonium chloride. Stirring was continued for another thirty minutes after the disappearance of colour; the liquid ammonia was allowed to evaporate overnight.

After about sixteen hours eleven grams of a white solid, giving a positive test for the sulfhydryl group, were found in the evaporating dish. The material began to char at about 250°, but did not melt up to 300°. It was washed twice with 25 ml. portions of ether giving 10.5 g. of a white solid. This material was dissolved in 150 ml. of cold water; and some undissolved solid (about 5mg.) was filtered off. The pH of the light yellow, strongly basic filtrate was adjusted to eight with 6 N hydrochloric acid, and after addition of a few mg. of ferric chloride the now dark purple solution was aerated overnight. When tested it proved negative for the sulfhydryl group.

On evaporation of the solution to a small volume on the steam bath seven grams (75%, based on the disodium salt) of a white, amorphous solid precipitated. This was dissolved in 80 ml. of 50% ethanol, boiled with a little Norit decolourizing charcoal, and refrigerated. Since, however, crystallization could not be effected in this manner, the solution was allowed to evaporate slowly at room tempera-

ture; a white solid (5.2 g.) precipitated from the solution, m.p. greater than 300° (charring starts at 250°; at 300° the solid has turned a dark brown colour). From the filtrate another gram of solid was recovered.

Anal. Calcd. for $C_6 H_{24} O_4 N_2 S_2$:
C,44.40; H,7.45; N,8.67; S,19.75.
Found: C,34.64; H,6.15; N,8.13; S,18.64.

The analysis shows that the compound could not have been free amino acid, a conclusion supported by its high solubility in water. Since the oxidation reaction was carried out at a pH of 8, it was concluded that the compound isolated was the sodium salt of α-ethyl-DL-cystine, for which

Anal. Calcd. for $C_{12} H_{24} O_4 N_2 S_2 Na_2$:
C,35.06; H,5.87; N,8.18; S,18.73.

The Synthesis of α-n-Propyl-DL-Cystine

Nitrosomethyl Urea (VI)[71]

In a two litre round bottom flask methylamine (292 g. -30-35%, Fisher certified reagent), concentrated hydrochloric acid (Fisher certified reagent, 225 ml., added, using methyl red as an indicator), urea (600 g., Fisher certified reagent), and water (420 ml.) were heated gently at reflux temperature for 2 3/4 hours and then vigorously for one

71 F. Arnd, in Organic Syntheses., ed. A. H. Blatt (New York, 1957), Coll. Vol. II, p. 461.

half hour. After a short period of heating the solution turned from red to yellow. It was left at room temperature overnight.

Sodium nitrite (220 g.-3 mole, 97%, Fisher certified reagent) was dissolved in the yellow liquid, the solution cooled to -15° in a dry ice-acetone bath and added slowly with stirring to a cooled mixture (-15°) of crushed ice (1200 g.) and concentrated sulfuric acid (200 g.). The temperature was maintained below 0° throughout the operation. A foamy, crystalline, white precipitate formed. After all of the ice in the mixture had melted, it was filtered with suction. The solid was then pressed on the filter, stirred to a paste with 100 ml. of cold water, partially dried in air, and then completely in a vacuum dessicator. The final product was stored in the refrigerator.

The yield is 210 - 230 g. (66 - 71%).

Diazomethane (VII)[72]

To a mixture of 40% potassium hydroxide (200 ml., Fisher certified reagent potassium hydroxide) and ether (500 ml., Fisher reagent chemical) nitrosomethylurea (60 g.) was added with stirring (magnetic stirrer) at 5° over a period of 10 minutes. The mixture was then stirred for another 15 minutes, the dark yellow ether layer was separated, and dried over potassium hydroxide pellets for three hours. The ether was then decanted.

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72 F. Arndt, op cit., p. 165.

Chloromethyl-n-Propyl Ketone (VIII)[73,74]

Butyryl chloride (12.5 cc. - 12.8 g., Eastman Organic Chemicals) was added slowly with shaking to the ethereal diazomethane solution in an ice bath. The solution decolorized and a gas was given off rapidly. After standing at room temperature for six hours a white solid appeared. The slow addition of concentrated hydrochloric acid to this mixture was accompanied by gas evolution. After half an hour at room temperature, the light yellow ether layer was separated from the darker acid phase, washed three times with small quantities of water, and dried over anhydrous sodium sulfate for two hours. The ether was then evaporated on the solvent stripper. Distillation of the yellow liquid at atmospheric pressure (750 mm.) yielded 6.5 g. (45%) of a nearly colourless, lachrymatory liquid, b.p. 152 - 156°, which was refrigerated after addition of a little magnesium oxide.

Benzylmercaptomethyl-n-Propyl Ketone (IX)[75,76]

The sodium salt of α-toluenethiol was prepared by extracting the latter (130 g.) in ether (500 cc.) with 10% sodium hydroxide solution (400 g.). The mixture was shaken several times while standing at room temperature for two

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73 J. R. Catch, op. cit., p. 278.

74 F. B. Reid, J. Org. Chem., 16, 1566 (1951).

75 R. Wahl, loc. cit.

76 K. T. Potts, loc. cit.

hours. The aqueous phase was then separated and heated at reflux temperature with the chloroketone (60 g.) (VIII) overnight. The non-aqueous light yellow top phase was washed with twenty-five ml. portions of water until free of chloride ions. The heavy oil thus obtained (101 g. - 97%) was dried over anhydrous sodium sulfate. Fractioned distillation gave a nearly colourless oil (86.5 g.) of boiling point 163 - 166° (9.5 mm.).

Anal. Calcd. for $C_{12}H_{16}OS$:

C,69.19; H,7.74; S,15.39.

Found: C,69.17; H,7.90; S,15.53.

5-Benzylmercaptomethyl-5-n-Propyl Hydantoin (X)[77,78]

Benzylmercaptomethyl n-propyl ketone (90 g.), potassium cyanide (40 g.), and ammonium carbonate (135 g.) in 50% ethanol (400 ml.) were added in a 1000 ml. three-necked round bottom flask equipped with a condensor and mechanical stirrer and heated on a water bath at 80 - 90° for seven hours. The dark yellow solution was allowed to evaporate at room temperature and normal pressure overnight and poured on crushed ice. The precipitated white solid was filtered with suction, washed with water and ether and dried in a vacuum dessicator. This procedure gave the hydantoin in 97% yield (52 g.), m.p. 148 - 151°. After one

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77 K. T. Potts, lic. cit.

78 I. W. Stapleton and J. M. Swan, loc. cit.

crystallization from hot ethanol 48 g. of white plates, m.p. 154°, was obtained.

Anal. Calcd. for $C_{14}H_{18}O_2N_2S$:
C,60.37; H,6.51; N,10.11; S,11.51.
Found: C,60.50; H,6.74; N,10.17; S,11.70.

S-Benzyl-α-n-Propyl-DL-Cysteine (XI)[79]

Hydantoin (X) (60 g.), barium hydroxide (315 g.), and water (1500 ml.) were heated at reflux temperature for forty hours in a two litre round bottom flask. Barium was precipitated as barium carbonate at 100° using carbon dioxide gas generated from marble chips and 6 N hydrochloric acid. The mixture was filtered while hot, the solid on the filter paper was washed with hot water and acetone, and the filtrate was allowed to cool, when 13 g. of unaltered hydantoin precipitated out. The filtrate was then left at room temperature overnight; after about sixteen hours thirty-nine gram (72%) of a white, amorphous solid, m.p. 218 - 220°, had come out of solution and was filtered with suction, washed with acetone, and dried on the filter. After crystallization from hot water, washing with acetone, and drying over phosphorus pentoxide in a vacuum dessicator to constant weight, the melting point of the product is 229°(the solid starts to turn brown at 220°). The compound is partly soluble in hot water, but insoluble in cold water, acetone, or ether.

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79 K. T. Potts, loc. cit.

Anal. Calcd. for $C_{13}H_{19}O_2NS$:

C,61.61; H,7.56; N,5.56; S,12.65.

Found: C,61.47; H,7.62; N,5.56; S,12.87.

α-n-Propyl-DL-Cystine (XII)[80,81]

Sodium metal (7.5 g.) cut into small pieces and S-benzyl-α-n-propyl cysteine (25 g.) were added alternatively in small amounts, while stirring, over a period of about thirty minutes to liquid ammonia (about 500 ml.). After stirring for sixty minutes the blue colour of the liquid was discharged with ammonium chloride (about 3.5 g.). The mixture (some white solid had precipitated out) was left at room temperature overnight to allow the ammonia to evaporate.

The white paste found in the evaporating dish was dissolved in cold water (350 ml.) and the mixture was filtered; the filtrate was then extracted with ether (35 ml.). The pH was adjusted to 7.5 with a few ml. of concentrated hydrochloric acid and 5 mg. of ferric chloride was added. The dark violet solution was aerated for thirty hours, at the end of which time the test for the sulfhydryl group was negative. The pH was then adjusted to six.

A light yellow, amorphous solid (11 g., 72%) precipitated when the solution was evaporated on the steam bath to about 200 ml.; this material was filtered off and washed with water and absolute ethanol. It began to char at 230°

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80 H. R. V. Arnstein, loc. cit.

81 J. L. Wood and V. du Vigneaud, loc. cit.

and had turned a very dark brown at 300°.

The solid was crystallized from boiling water (about 1000 ml.) containing a few mg. of decolorizing charcoal. As the filtrate was evaporated on a steambath, plates of a crystalline, white solid appeared first on the liquid surface.

The yield of crystalline mats of needles is 6.5 g., m.p.$\geq$ 300° (chars at 290°).

Anal. Calcd. for $C_{12}H_{24}O_4N_2S_2$:
C,44.42; H,7.46; N,8.63.
Found: C,44.11; H,7.54; N,8.50.

The filtrate, from which 11 g. of product had been recovered, was evaporated further on the steam bath to about 50 ml. when another crop of a white crystalline solid appeared. Upon filtration four gram of product were obtained.

The solid gives a deep red colour with a few drops for 5% sodium nitroprusside solution and a few drops of 10% sodium hydroxide solution. It is quite soluble in water. The solid was dissolved in hot water, boiled with Norit decolorizing charcoal, and filtered; on cooling of the filtrate 3 g. of white needles of crude amino acid, m.p.$>$300°, was obtained.

All elementary analyses were carried out by Schwarzkopf Microanalytical Laboratory, 56-19 37th Avenue, Woodside 77, N. Y.

TABLE I

SOME CHARACTERISTICS OF NEW

COMPOUNDS PREPARED

Compound	Melting Point (°C)	Boiling Point (°C)	Yield (%)
(II)		126-128 (3.5mm.)	98
(III)	130-131		90
(IV)	224-226		74
(V)	>300		75[a] (49[b])
(IX)		163-166 (9.5mm.)	97
(X)	154		97
(XI)	229		72
(XII)	>300		72 (49[c])

[a] Yield based on the disodium salt of α-ethyl-DL -cystine.

[b] Overall yield based on the halo ketone as starting material and considering only crude yields.

[c] Overall yield based on the halo ketone as starting material and considering only crude yields.

Discussion

The α-halogenation of ketones was carried out in two different ways: by the halogenation of the corresponding ketone and by acid decomposition of the diazo ketone formed from the ketone and diazomethane. In the first case separation by fractional distillation of the two isomers formed by bromination of methyl ethyl ketone was found very time consuming. Yields of the desired isomer, bromomethyl ethyl ketone, were small compared to the other isomer obtained. The separation was not as efficient as reported in the literature,[82] because the apparatus used was less elaborate. The second method did not require a fractional distillation, but the preparation of the requisite amount of ketone was also time consuming. A large quantity of starting material was required, since the overall yield of amino acid was fifty percent. This yield could probably be improved by a closer study of all the reaction conditions, particularly those of the hydrolysis of hydantoins and the reduction of S-benzyl amino acids.

The haloketones turned from a very light yellow colour to a light green on standing for about a week, in spite of the presence of magnesium oxide. This indicates some decomposition and loss of hydrochloric or hydrobromic acid. The ketones should therefore be used as quickly as possible after preparation.

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82 J. R. Catch, J. Chem. Soc., 272 (1948).

The condensation of the sodium salt of α-toluenethiol with α-haloketone proceeds smoothly and gives a high yield of the benzylmercapto ketone. The Bucherer-Bergs synthesis of hydantoin in 50% alcohol gives a crystalline product in good yield. After hydrolysis by barium hydroxide a few gram of unreacted hydantoin had to be separated from the S-benzyl amino acid. Washing of the precipitated barium carbonate with hot water recovered a small amount of S-benzyl amino acid.

The removal of the benzyl group by sodium in liquid ammonia is a fast reaction. The free amino acid can be obtained this way after oxidation, if the pH is adjusted to six; at a pH of eight one gets the sodium salt instead. This is very soluble in water and therefore hard to separate from inorganic material. Precipitation at the isoelectric point yields the free amino acid from the salt.

This synthesis of alpha substituted cystines should be applicable also when the substituent is a large, bulky group rather than the simple alkyl chain. Some difficulties might occur in the preparation of hydantoins in this case, because of steric hindrance. However, 5-substituted hydantoins such as 5-benzylmercaptomethyl-5-phenyl hydantoin have been prepared in this manner.[83]

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83 L. M. Long, U. S., 2,404,509. (c.f. C. A., 40, 6095 (1946)).

CHAPTER IV.

SUMMARY AND CONCLUSIONS

An attempt was made to synthesize α-phenylalanine by the decomposition of α-cyano-α-phenylpropionylazide in various reaction media and hydrolysis of the decomposition product.

The compound obtained by decomposition of the azide in anhydrous ethanol saturated with dry hydrogen chloride could not be identified. The elementary analysis of it excludes the corresponding urethane, isocyanate, amino nitrile, or disubstituted urea, which could theoretically form in the reaction.

During the decomposition either a quantitative amount of gas or nearly twice the theoretical quantity was given off; gas evolution was not consistent.

When trifluoroacetic acid was used as the reaction medium, the products of the reaction were the hydrazide in about forty percent yield, a smaller amount of a solid of lower melting point, and a small quantity of an oil. Use of a mixture of the acid and its anhydride or the acid and a small percentage of water gave the same final products. When the azide and the acid were heated under reflux for several hours the same products were obtained. Because of the very small yields little effort

was made to identify the minor products of the reaction.

Hydrolysis of the product of the decomposition in ethanol gave a material, which has the properties of an amino acid and a melting point identical with that of α-phenylalanine, in small yields.

Elementary analysis of the azide showed a difference from the theoretical composition. This could be the result of partial decomposition in the medium, dimethylformamide and methanol, used for crystallization. However, the possibility that the azide was not formed from the hydrazide cannot be completely discarded. The azide should be prepared by a different method. It might prove rewarding to identify every reaction product in order to understand the mechanisms involved.

The alpha substituted cystines, α-ethyl-DL-cystine and α-n-propyl-DL-cystine were prepared making use of a hydantoin as an intermediate. α-Haloketones needed as starting material were either prepared by the halogenation of a ketone or by the reaction of an acid chloride with diazomethane and decomposition of the resulting diazoketone with mineral acid. Condensation of the α-haloketones with the sodium salt of α-toluenethiol gave the benzylmercaptomethyl ketones. The hydantoins were formed from the latter by way of the Bucherer-Bergs Synthesis in which the ketone is heated with potassium cyanide and ammonium carbonate in fifty percent alcohol. The hydantoins were hydrolysed with barium hydroxide to give the S-benzyl amino

acids. The benzyl group was then removed by reduction in sodium in liquid ammonia solution. ∝-Ethyl-DL-cystine was obtained as the sodium salt by oxidation at a pH of eight in the presence of a trace of ferric chloride; ∝-n-propyl -DL-cystine was prepared as the free amino acid by changing the pH to six after oxidation.

The synthesis of alpha substituted cystines by way of the hydantoin is a useful method of preparation. The overall yield of amino acid of fifty percent compares well with other syntheses of amino acids such as the Strecker Synthesis or Curtius reaction. A similar synthesis of alpha substituted cystines containing groups, which produce some steric hindrance, should also be feasible.

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